PB82-246836

Evaluation of Gel Permeation Chromatography for Clean Up of Human Adipose Tissue Samples for GC/MS Analysis of Pesticides and Other Chemicals

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

28 Dec 81

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TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA-600/J-82-005	2. JOURNAL ARTICLE	3. RE PB82-246836
4. TITLE AND SUBTITLE Evaluation of Gel Permeation Chromatography for Clean Up of Human Adipose Tissue Samples for GC/MS Analysis of Pesticides and Other Chemicals		5. REPORT DATE 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Kathryn E. MacLeod, Robert C. Hanisch, and Robert G. Lewis		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Analytical Chemistry Branch Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711		10. PROGRAM ELEMENT NO. ACAE1A 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Office of Research and Development (RTP,NC) Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Journal of Analytical Toxicology 6: 38-40, 1982

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17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

Journal of Analytical Toxicology, Vol. 6, January/February 1982

EPA-600/J-82-005
JOURNAL ARTICLE

Evaluation of Gel Permeation Chromatography for Clean up of Human Adipose Tissue Samples for GC/MS Analysis of Pesticides and Other Chemicals

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Abstract

Gel permeation chromatography (GPC) has been evaluated for clean up of human adipose tissue sample extracts in preparation for confirmatory analysis by gas chromatography-mass spectrometry (GC/MS). Studies were conducted with standard solutions, fortified chicken fat, and actual human adipose tissue samples. Nearly quantitative removal of lipid material was achieved with minimal losses of 16 organochlorine pesticides and metabolites: two polychlorinated biphenyl (PCB) mixtures, two chlorinated napthalene (PCN) mixtures, and a polybrominated biphenyl (PBB) mixture. Mass spectra free from interferences were obtained over the mass range from 46 m/z to 560 m/z.

Introduction

Earlier, this laboratory reported (1) the first application of gel permeation chromatography (GPC) for clean up of human adipose tissue samples for analysis by combined gas chromatography-mass spectrometry (GC/MS). Since that time, the method has been evaluated for separation of several commonly-occurring organochlorine (OC) pesticides and metabolites from human adipose tissues. Also examined were two commercial polybichlorinated biphenyl (PCB) mixtures, two polychlorinated naphthalenes (PCNs) and a polybrominated biphenyl (PBB) mixture.

The GPC methodology was studied for application to tissue specimens collected by the U.S. Environmental Protection Agency under its National Human Monitoring Program (2). As a part of that program, adipose samples weighing from 1 to 10 g were taken for individual analysis, and composited extracts from Florisil column chromatographic clean up (3) were sent to this laboratory for confirmatory analysis by GC/MS. These composite samples contained up to several grams of lipid material from which chemical residues must be separated for analysis. The purpose of this study was to determine if GPC could be used to quantitatively recover the compounds of interest from the tissue extracts with minimal interference to GC/MS analysis.

Experimental

Gel Permeation Chromatography

The GPC system consisted of A) a 45 cm x 15 mm i.d. chromatographic column (5820-20, Ace Glass, Vineland, New Jersey); B) a feed tube adapter (5827-24); C) a 500 mL reservoir (5824-10); D) a bottom drip adapter with Teflon® stopcock (5835-B-11); E) two nylon couplings (5840-10); F) pressurized nitrogen with suitable regulator; G) a needle valve to regulate the flow of nitrogen into the system; H) copper tubing from the nitrogen regulator to the needle valve; and I) heat shrink tubing to connect the needle valve to the feed tube adapter. The gel material was Bio-Beads SX-2 (Bio-Rad Laboratories, Richmond, California). Cyclohexane was used as the elutant.

Gas Chromatography

All GC analyses were performed on a Tracor (Austin, Texas) Model 222 instrument equipped with a pulsed linearized ^{63}Ni electron capture detector (ECD). Two 183 cm x 4 mm i.d. borosilicate glass columns were used: a 1.5% OV-17/1.95% OV-210 on 80/100 mesh Gas Chrom Q and 4% SE-30/6% OV-210 on 80/100 mesh Gas Chrom Q. Both were operated at 200°C, with nitrogen carrier gas at 70 mL and 85 mL/min, respectively.

Mass Spectrometry

A Hewlett-Packard (Palo Alto, California) Model 5930A mass spectrometer was operated at 70 eV and 120 μamp filament emission. The ion source temperature was maintained at 180°C and the mass filter temperature at 110°C. Data were collected, stored, and plotted using a Hewlett-Packard Model 5932A data system. A Hewlett-Packard Model 5700A gas chro-

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matograph was interfaced with the mass spectrometer and equipped with a 183 cm x 2 mm i.d. borosilicate glass column packed with 1.5% OV-17/1.95% OV-210 on 80/100 mesh Gas Chrom Q. Temperature programmed runs were made at 80°C (2 min) to 210°C at 8°C/min. Helium flow was 40 mL/min.

Reagents and Standards

Cyclohexane, hexane, acetonitrile, and petroleum ether were glass distilled from Burdick and Jackson (Muskegon, Michigan). Diethyl ether (containing 2% ethanol) was AR grade from Mallinckrodt (St. Louis, Missouri). Pesticides, PCBs, and PCNs, were obtained from the Analytical Reference Standards Repository, U.S. E.P.A. (Research Triangle Park, North Carolina). The PBB used was fireMaster BP-6 from Michigan Chemical Corporation (Chicago, Illinois). The mixture consists of about 63% hexabromobiphenyls, 33% heptachlorobiphenyls, and 4% pentachlorobiphenyls (4). The principal component is reported to be 2,4,5,2',4',5'-hexabromobiphenyl at 56% (4).

Results and Discussion

A standard solution in 100 mL cyclohexane was prepared containing the 16 common OC pesticides and metabolites shown in Table I. This mixture was subjected to GPC alone and in combination with 5 mL aliquots of a solution of 0.1 g/mL rendered chicken fat in cyclohexane to establish recoveries and elution volume. Fractions as small as 10 mL were collected for individual analysis by GC/ECD. Results shown in Table I indicate good separation with a 45 cm x 15 mm i.d. column containing 30.5 g Bio-Beads SX-2 operated at 2 to 5.5 mL/min elution rate (swelling factor for cyclohexane 2.61 cm^3/g). The first 60 mL of eluate contained an average of 95% of the lipid material from the fat extract and was designated as the dump fraction. The fraction also contained 1 to 6% of the mirex, but less than 1% of any of the other pesticides or metabolites. A 75 mL dump fraction contained an average of 99.4% of the lipid and 6 to 12% of the mirex.

Table I. Recovery and Elution Data for 16 Pesticides and Metabolites

Pesticide	Fortification Level, μg	Average Recovery,* %	Relative Standard Deviation	Elution Volume, mL
α-HCH	0.2	105	14	70-125
Lindane	0.2	93	5	105-135
Heptachlor	0.2	91	8	70- 95
Oxychlordane	0.3	99	9	70- 95
trans-Nonachlor	1.0	102	6	70- 95
Mirex	2.0	96	8	55- 95
β-HCH	0.8	96	8	250-350
Aldrin	0.4	89	9	70- 95
Heptachlor epoxide	0.6	100	6	70-115
p,p'-DDE	0.8	112	9	70-105
Dieldrin	1.0	102	6	85-115
o,p'-DDD	1.6	99	6	85-125
Endrin	1.6	100	6	70-115
o,p'-DDT	1.8	98	4	70-105
p,p'-DDD	1.6	98	7	105-160
p,p'-DDT	2.0	96	5	85-115

*Average of 4 replicate determinations.

Mirex exhibited the smallest elution volume and was removed quantitatively by the first 100 mL of elutant. Most of the remaining OC pesticides and metabolites began eluting after a volume of 70 to 85 mL. β-Hexachlorocyclohexane (β-HCH), which has the smallest molecular volume of the compounds tested, did not begin to elute until 205 mL of solvent had passed through the column. Aldrin gave consistently low and *p,p'*-DDE gave consistently high recoveries.

No change in elution behavior was observed when the chicken fat solution and standard mixture were placed on the column simultaneously. Early fractions following the dump fraction were slightly yellow in color, but contained no measurable lipid residue. It is thought that the color was imparted by carotene, a yellow pigment found in adipose tissue.

The methodology was further validated by analysis of a human fat sample, which was fortified to contain 0.9 ppm β-HCH, 0.6 ppm oxychlordane, 7.7 ppm *p,p'*-DDE, and 1.5 ppm *p,p'*-DDT. (Concentrations include background levels of 0.4, 0.1, 3.8, and 0.5 ppm for these compounds, respectively.) A 6.1-g sample of this tissue was dissolved in 100 mL cyclohexane and 5 mL aliquots (lipid weight 0.3 g) were subjected to GPC as before. Elution patterns were identical to those observed with chicken fat; total recoveries were: β-HCH, 96%; oxychlordane, 134%; *p,p'*-DDE, 109%; and *p,p'*-DDT, 85%.

Two PCB mixtures (Aroclor 1254 and Aroclor 1260, Monsanto, St. Louis, Missouri), two PCN mixtures (Halowax 1051 and 1099, Koppers Co., Pittsburgh, Pennsylvania), and one PBB mixture (fireMaster BP-6) were also examined in standard solutions and in human adipose tissue extracts. The PCBs were eluted in the early fractions (70-125 mL) and were recovered quantitatively (avg. 109%). The heptachlorobiphenyl congeners, which coelute with mirex on the GC columns used in this study (5), began elution at 75 mL and could be partially separated from mirex by careful fractionation. The presence or absence of mirex in a particular fraction was confirmed by the method of Lewis *et al.* (5).

As expected, the PCNs coeluted with the PCBs. Recoveries of the PCNs averaged 114% in the 70 to 125 mL fraction. A total recovery of only 85% was attained for the PBB, with 93% of the total eluting in the 50 to 150 mL fraction.

A composite sample of human adipose tissue extracts (15% diethyl ether in hexane fraction from Florisil) was selected to evaluate the GPC procedure for GC/MS analyses. The sample, which contained 0.5 g of lipid, was dissolved in 5 mL cyclohexane and placed on the column. After a 70 mL dump fraction, 425 mL of eluate was collected for analysis. The eluate was concentrated first under vacuum in a rotary evaporator and finally under a gentle stream of nitrogen at room temperature to 0.5 mL for GC/MS analysis.

A typical mass spectrum shown in Figure 1 illustrates the benefit derived from the GPC clean up of a sample of composited fractions from the Florisil chromatography of human adipose tissue extracts. The lower (and more abundant) mass ions of dieldrin, m/z 79, 82, and 107, are clearly visible and readily measurable. In samples not subjected to GPC clean up, the low-mass region is generally so cluttered that it is fruitless to scan below 100 to 130 m/z. The M^+ and M^+-Cl ions are also clearly discernible after GPC clean up, whereas they are often obscured otherwise. With GPC clean up, the mass spectra were free of serious interference from 46 to 560 m/z.

For several years, this laboratory has successfully applied GPC to the clean up of human adipose tissue extracts for GC/MS confirmation of a wide variety of pesticides and their

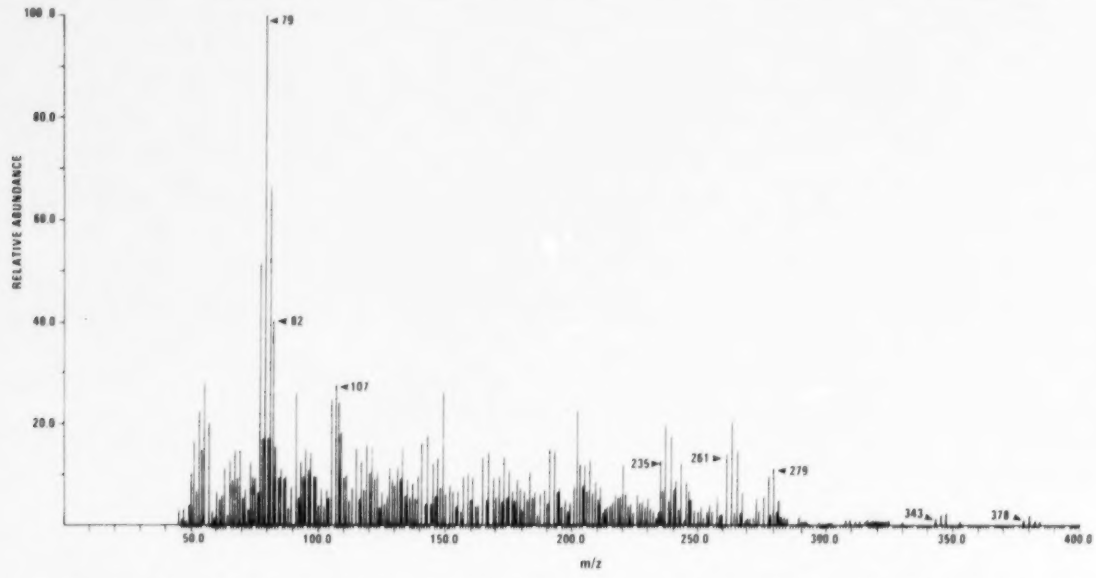

Figure 1. Mass spectrum of dieldrin in a composite human adipose tissue extract (15% diethyl ether in hexane from Florisil chromatography) after GPC clean up.

metabolites and of many halogenated organic chemicals and phthalates. When necessary, two consecutive passes of the extract through the GPC column may be made without significant losses of the particular chemical. For example, a sample containing 500 mg of lipid before clean up will yield one containing less than 1 mg in two passes when the dump fraction is 60 mL. Increasing the dump fraction to 75 mL (when mirex is absent), would further reduce the lipid residue to 0.02 mg.

Acknowledgement

The authors wish to thank G.W. Sovocool and L.E. Feige for the mass spectrometric analyses.

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Manuscript received September 25, 1981;
revision received December 28, 1981.